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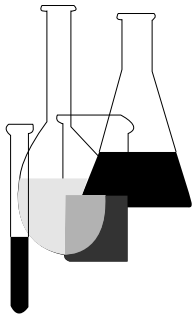
Health Effects Test Guidelines

OPPTS 870.5195 Mouse Biochemical Specific Locus Test



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5195 Mouse biochemical specific locus test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5195 Mouse biochemical specific locus test and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** The mouse biochemical specific locus test (MBSL) may be used to detect and quantitate mutations originating in the germ line of a mammalian species.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Biochemical specific locus mutation is a genetic change resulting from a DNA lesion causing alterations in proteins that can be detected by electrophoretic methods.

Germ line comprises the cells in the gonads of higher eukaryotes, which are the carriers of the genetic information for the species.

(d) **Test method**—(1) **Principle.** The principle of the MBSL is that heritable damage to the genome can be detected by electrophoretic analysis of proteins in the tissues of the progeny of mice treated with germ cell mutagens.

(2) **Description.** For technical reasons, males rather than females are generally treated with the test chemical. Treated males are then mated to untreated females to produce F_1 progeny. Both blood and kidney samples are taken from progeny for electrophoretic analysis. Up to 33 loci can be examined by starch-gel electrophoresis and broad-range isoelectric focussing. Mutants are identified by variations from the normal electrophoretic pattern. Presumed mutants are bred to confirm the genetic nature of the change.

(3) **Animal selection**—(i) **Species and strain.** Mice should be used as the test species. Although the biochemical specific locus test could be performed in a number of inbred strains, in the most frequently used cross, C57BL/6 females are mated to DBA/2 males to produce (C57BL/6 $\times$ DBA/2) F_1 progeny.

(ii) **Age.** Healthy, sexually mature (at least 8 weeks old) animals should be used for treatment and breeding.

(iii) **Number.** A decision on the minimum number of treated animals should take into account possible effects of the test chemical on the fertility of the treated animals. Other considerations should include:

(A) The production of concurrent spontaneous controls.

(B) The use of positive controls.

(C) The power of the test.

(4) **Control groups**—(i) **Concurrent controls.** An appropriate number of concurrent control loci should be analyzed in each experiment. These should be partly derived from matings of untreated animals (from 5 to 20 percent of the treated matings), although some data on control loci can be taken from the study of the alleles transmitted from the untreated parent in the experimental cross. However, any laboratory which has had no prior experience with the test should produce a spontaneous control sample of about 5,000 progeny animals and a positive control sample (using 100 mg/kg ethylnitrosourea) of at least 1,200 offspring.

(ii) **Historical controls.** Long-term, accumulated spontaneous control data (currently, 1 mutation in 1,200,000 control loci screened) are available for comparative purposes.

(5) **Test chemicals—**(i) **Vehicle.** When possible, test chemicals should be dissolved or suspended in distilled water or buffered isotonic saline. Water-insoluble chemicals should be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test chemical nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

(ii) **Dose levels**. Usually, only one dose need be tested. This should be the maximum tolerated dose (MTD), the highest dose tolerated without toxic effects. Any temporary sterility induced due to elimination of spermatogonia at this dose must be of only moderate duration, as determined by a return of males to fertility within 80 days after treatment. For evaluation of dose-response, it is recommended that at least two dose levels be tested.

(iii) **Route of administration.** Acceptable routes of administration include, but are not limited to, gavage, inhalation, and mixture with food or water, and intraperitoneal or intravenous injections.

(e) **Test performance**—(1) **Treatment and mating.** Male DBA/2 mice should be treated with the test chemical and mated to virgin C57BL/6 females immediately after cessation of treatment. Each treated male should be mated to new virgin C57BL/6 females each week. Each pairing will continue for a week until the next week's mating is to begin. This mating schedule permits sampling of all post-spermatogonial stages of germ-cell development during the first 7 weeks after exposure.

Spermatogonial stem cells are studied thereafter. Repeated mating cycles should be conducted until sufficient offspring have been obtained to meet the power criterion of the assay for spermatogonial stem cells.

(2) **Examination of offspring**—(i) **Birth and weaning.** Offspring should be examined at birth and at weaning for externally detectable changes in morphology and behavior; these could be due to dominant mutations. Such characteristics may include, but are not limited to, variations in coat color, appearance of eyes, size (in which case weighing of variant animals and littermates should be carried out), fur texture, etc. Gross changes in external form and behavior should also be sought. Scrutiny of such visible characteristics of all animals should be made during all subsequent manipulations of the animals.

(ii) **Tissue sampling**. Blood (about 0.1 mL) and one kidney should be removed from progeny mice under anesthesia. Both tissues are then prepared for analysis by electrophoresis.

(iii) **Electrophoresis**. The gene products of 6 loci should be analyzed in the blood sample by broad-range isoelectric focusing and of 27 loci in the kidney sample by starch-gel electrophoresis and enzyme-specific staining. Details on these procedures are included in paragraphs (h)(2) and (h)(3) of this guideline.

(iv) **Mutant identification**. Presumptive electrophoretic mutants should be identified by variation from the normal electrophoretic banding patterns. Reruns of all variant samples should be performed to confirm the presence of altered banding patterns. Samples from parents of progeny exhibiting banding pattern variations should be assayed to determine whether the variant was induced by the experimental treatment or was pre-existing. All treatment-induced variants are bred to determine the genetic nature of the change.

(f) **Data and reports**—(1) **Treatment of results.** Data should be presented in tabular form and should permit independent analysis of cell stage-specific effects, and dose-dependent phenomena. The data should be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected should be thoroughly described. In addition, concurrent positive control data (if employed) and spontaneous control data should also be tabulated. These concurrent controls should be added to, as well as compared with, the historical control data.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results**. (i) There are several criteria for determining a positive response, one of which is a statistically significant dose-related increase in the frequency of electrophoretic mutations. Another cri-

terion may be based upon detection of a reproducible and statistically significant positive response for at least one of these test points.

(ii) A test chemical which does not produce a statistically significant increase in the frequency of electrophoretic mutations over the spontaneous frequency, or a statistically significant and reproducible positive response for at least one of the test points, is considered nonmutagenic in this system, provided that the sample size is sufficient to exclude a biologically significant increase in mutation frequency.

(iii) Biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation**. (i) Positive results in the MBSL indicate that, under the test conditions, the test chemical induces heritable gene mutations in a mammalian species.

(ii) Negative results indicate that, under the test conditions, the test chemical does not induce heritable gene mutations in a mammalian species.

(5) **Test report**. In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Strain, age, and weight of animals used; numbers of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used, rationale for dose selection, and toxicity data, if available.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Number of loci screened for both treated and spontaneous data.

(vi) Criteria for scoring mutants.

(vii) Number of mutants found/locus.

(viii) Loci at which mutations were found.

(ix) Use of concurrent negative and positive controls.

(x) Dose-response relationship, if applicable.

(g) **Additional requirements.** Testing facilities conducting the mouse biochemical specific locus test in accordance with this section should, in addition to adhering to the provisions of 40 CFR 792.190 and 792.195, obtain, adequately identify and retain for at least 10 years, acceptable 35–mm photographs (and their negatives) of the stained isoelectric-focusing

columns and the stained starch-gels obtained following analyses of blood and kidney preparations, respectively, from mutant mice, their siblings, and their parents.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Johnson, F.M. et al. The detection of mutants in mice by electrophoresis: Results of a model induction experiment with procarbazine. *Genetics* 97:113–124 (1981).

(2) Johnson, F.M. and Lewis, S.E. Mutation rate determinations based on electrophoretic analysis of laboratory mice. *Mutation Research* 82:125–135 (1981).

(3) Johnson, F.M. and Lewis, S.E. Electrophoretically detected germinal mutations induced by ethylnitrosourea in the mouse. *Proceedings of the National Academy of Sciences* 78:3138–3141 (1981).

(4) Lewis, S.E. et al. Dominant visible and electrophoretically expressed mutations induced in male mice exposed to ethylene oxide by inhalation. *Environmental Mutagenesis* 8:867–872 (1986).